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Citation for published version:

Franzmeier, N, Ren, J, Damm, A, Monté-rubio, G, Boada, M, Ruiz, A, Ramirez, A, Jessen, F, Düzel, E, Rodríguez Gómez, O, Benzinger, T, Goate, A, Karch, CM, Fagan, AM, Mcdade, E, Buerger, K, Levin, J, Duering, M, Dichgans, M, Suárez-calvet, M, Haass, C, Gordon, BA, Lim, YY, Masters, CL, Janowitz, D, Catak, C, Wolfsguber, S, Wagner, M, Milz, E, Moreno-grau, S, Teipel, S, Grothe, MJ, Kilimann, I, Rossor, M, Fox, N, Laske, C, Chhatwal, J, Falkai, P, Perneczky, R, Lee, J, Spottke, A, Boecker, H, Brosseon, F, Fliessbach, K, Heneka, MT, Nestor, P, Peters, O, Fuentes, M, Menne, F, Priller, J, Spruth, EJ, Franke, C, Schneider, A, Westerteicher, C, Speck, O, Wiltfang, J, Bartels, C, Araque Caballero, MA, Metzger, C, Bittner, D, Salloway, S, Danek, A, Hassenstab, J, Yakushev, I, Schofield, PR, Morris, JC, Bateman, RJ & Ewers, M 2019, 'The BDNFVal66Met SNP modulates the association between beta-amyloid and hippocampal disconnection in Alzheimer's disease', *Molecular Psychiatry*. <https://doi.org/10.1038/s41380-019-0404-6>

Digital Object Identifier (DOI):

[10.1038/s41380-019-0404-6](https://doi.org/10.1038/s41380-019-0404-6)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Molecular Psychiatry

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The *BDNF*_{Val66Met} SNP modulates the association between beta-amyloid and hippocampal disconnection in Alzheimer's disease

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Study funding

This project was supported by The Dominantly Inherited Alzheimer's Network (DIAN, UF1 AG032438) funded by the National Institute on Aging (NIA), the German Center for Neurodegenerative Diseases (DZNE), the NIHR Queen Square Dementia Biomedical Research Centre and the MRC Dementias Platform UK (MR/L023784/1 and MR/009076/1), and AMED under Grant Number JP17dk0207036 and JP17kk0205009. This manuscript has been reviewed by DIAN Study investigators for scientific content and consistency of data interpretation with previous DIAN Study publications. We acknowledge the altruism of the participants and their families and contributions of the DIAN research and support staff at each of the participating sites for their contributions to this study.

The FACEHBI study is supported by Grifols®, Piramal®, Araclon Biotech®, Laboratorios Echevarne S.A. and Fundació ACE, Institut Català de Neurociències Aplicades.

M. Ewers, Alzheimer Forschung Initiative & LMUexcellent

J. Chhatwal – K23AG049087

B.A. Gordon – K01AG053474, Barnes-Jewish Hospital Foundation Willman Scholar Fund

Y. Lim – National Health and Medical Research Council (NHMRC) GNT1111603, GNT1147465

Disclosures:

A.M. Fagan - A.M.F. has received research funding from Biogen, Fujirebio and Roche Diagnostics. She is a member of the scientific advisory boards for Roche, Genentech and AbbVie and also consults for Araclon/Griffols and DiamiR

Y. Lim – has served as a scientific consultant to Biogen and Lundbeck

M. Boada who has consulted or advisory board for Araclon, Grifols, Lilly, Nutricia, Roche and Servier. She received fees for lectures and funds for research from Araclon, Grifols, Nutricia, Roche and Servier. She has not received personal compensations from these organizations. Agustín Ruiz has consulted for Grifols and Landsteiner Genmed. He received funds for research and/or reimbursement of expenses for congresses attendance from Araclon, and Grifols. He has not received personal compensations from these organizations.

T. Benzinger – Investigator initiated research funding sponsored by Avid Radiopharmaceuticals (a wholly owned subsidiary of Eli Lilly), and Foundation for the NIH. Clinical trials sponsored by Avid Radiopharmaceuticals, Eli Lilly, Roche, Jaansen, Biogen, and NIH. Travel sponsored by the American Society for Neuroradiology, Alzheimer's Association International Convention, NIH.

Conflicts of interest statement

The authors declare that there is no conflict of interest to disclose

ABSTRACT

In Alzheimer's disease (AD), a single nucleotide polymorphism in the gene encoding brain-derived neurotrophic factor (*BDNF*_{Val66Met}) is associated with worse impact of primary AD pathology (beta-amyloid, A β) on neurodegeneration and cognitive decline, rendering *BDNF*_{Val66Met} an important modulating factor of cognitive impairment in AD. However, the effect of *BDNF*_{Val66Met} on functional networks that may underlie cognitive impairment in AD is poorly understood. Using a cross-validation approach, we first explored in subjects with autosomal dominant AD (ADAD) from the Dominantly Inherited Alzheimer Network (DIAN) the effect of *BDNF*_{Val66Met} on resting-state fMRI assessed functional networks. In seed-based connectivity analysis of six major large-scale networks, we found a stronger decrease of hippocampus (seed) to medial-frontal connectivity in the *BDNF*_{Val66Met}-carriers compared to *BDNF*_{Val}-homozygotes. *BDNF*_{Val66Met} was not associated with connectivity in any other networks. Next, we tested whether the finding of more pronounced decrease in hippocampal-medial-frontal connectivity in *BDNF*_{Val66Met} could be also found in elderly subjects with sporadically occurring A β , including a group with subjective cognitive decline (N=149, FACEHBI study) and a group ranging from preclinical to AD dementia (N=114, DELCODE study). In both of these independently recruited groups, *BDNF*_{Val66Met} was associated with a stronger effect of more abnormal A β -levels (assessed by biofluid-assay or amyloid-PET) on hippocampal-medial-frontal connectivity decreases, controlled for hippocampus volume and other confounds. Lower hippocampal-medial-frontal connectivity was associated with lower global cognitive performance in the DIAN and DELCODE studies. Together these results suggest that *BDNF*_{Val66Met} is selectively associated with a higher vulnerability of hippocampus-frontal connectivity to primary AD pathology, resulting in greater AD-related cognitive impairment.

INTRODUCTION

Brain derived neurotrophic factor (BDNF) is part of the neurotrophin family, playing a critical role in synaptic plasticity and repair throughout the lifespan.¹⁻³ BDNF is expressed beyond the neocortex particularly in the hippocampus,⁴ where BDNF is essential for long-term potentiation (LTP) underlying hippocampus-related memory.^{1, 3, 5} Hippocampus-dependent memory impairment is a key feature of Alzheimer's disease (AD), where beta-amyloid (A β) and tau are hallmark pathologies affecting the hippocampus early in AD.⁶⁻⁸ Post-mortem studies showed decreased hippocampal BDNF-levels among other brain regions in patients with AD dementia or mild cognitive impairment (MCI).^{9, 10} These observations raise the important question whether altered BDNF-levels modulate the impact of AD pathology on brain function. Preclinical research in transgenic AD mouse models showed that genetic delivery or overexpression of BDNF improved hippocampus LTP^{11, 12} and alleviated the impact of amyloid or tau pathology on cell loss and memory,^{12, 13} suggesting protective BDNF-effects in AD. However, in humans the role of BDNF in modulating the impact of AD pathology is not well understood.

A single nucleotide polymorphism (SNP) (rs6265) resulting in the substitution of Valine by Methionine in the BDNF pro-domain (i.e. *BDNF*_{Val66Met}) leads to reduced synaptic BDNF release.^{14, 15} Because *BDNF*_{Val66Met} shows a relatively high prevalence ranging from 29% in European to 72% in Asian countries,¹⁶ *BDNF*_{Val66Met} may constitute a genetic factor that modifies functional brain alterations in a substantial portion of AD subjects.¹⁷⁻²¹ Although neuroimaging studies in AD showed only small *BDNF*_{Val66Met}-effects on hippocampal volume,^{22, 23} several studies have found *BDNF*_{Val66Met}-related reductions in hippocampal FDG-PET metabolism and stronger memory impairment in both autosomal dominant and sporadic AD.^{17, 19-21} In line with the results in transgenic AD mouse models,^{12, 13} these results suggest that *BDNF*_{Val66Met} influences in particular the impact of AD pathology on hippocampal function and memory. Yet, the impact of *BDNF*_{Val66Met} at the level of functional networks that

support cognitive function is unknown. Thus, our major aim was to assess whether *BDNF*_{Val66Met} moderates the impact of AD pathology on functional connectivity within major networks and associated cognitive impairment in AD.

The brain is composed of major large-scale functional networks that become engaged during cognitive tasks. Resting-state fMRI studies assessing the integrity of these networks in AD have shown reduced connectivity between the hippocampus and medial-frontal and posterior-parietal regions of the default-mode network (DMN).²⁴⁻²⁸ Higher A β -levels were associated with lower hippocampus connectivity underlying memory impairment in preclinical and prodromal AD patients.^{29, 30} Here, we hypothesized that in *BDNF*_{Val66Met} carriers, the impact of higher A β pathology on hippocampus connectivity and memory impairment is worse compared to homozygous *BDNF*_{Val}-carriers. We tested this hypothesis in three independently recruited samples of subjects with either genetically caused AD or biomarker evidence of A β . In a discovery sample of 115 mutation-carriers with autosomal dominant AD (ADAD) and 91 familial non-carrier controls from the Dominantly Inherited Alzheimer Network (DIAN),³¹ we explored in which functional network *BDNF*_{Val66Met}-carriers showed altered connectivity compared to homozygous *BDNF*_{Val}-carriers.

For our hypothesis-driven analysis we assessed connectivity alterations in the hippocampal network, and for exploratory purposes, in a set of other major functional networks (i.e. DMN, dorsal attention [DAN], salience [SAL], fronto-parietal control [CON]) that are part of the canonical set of resting-state networks associated with higher cognitive function and previously found altered in AD.^{32, 33} We specifically conducted the discovery analyses in ADAD, since the study design of DIAN allows to examine the effect of *BDNF*_{Val66Met} on connectivity changes in genetically caused AD where aging-related comorbidities such as cerebrovascular changes are unlikely. Based on the results on the association between *BDNF*_{Val66Met} and functional network changes in ADAD, confirmatory analyses were subsequently conducted in elderly subjects with biomarker evidence of sporadic AD,

including 149 subjects with subjective cognitive decline (SCD) from the Spanish FACEHBI study³⁴ and in 114 subjects ranging from cognitively normal to AD dementia assessed within the German DELCODE study.³⁵ The inclusion of these additional samples with sporadic AD pathophysiology provided the opportunity to test whether any effects of *BDNF*_{Val66Met} on connectivity can be generalized towards the more common age-related form of accumulating AD pathology in elderly subjects. Since *BDNF*_{Val66Met} has been previously associated with greater cognitive impairment in both autosomal dominant and sporadic AD,^{17, 19, 20} we tested in a last step whether any observed *BDNF*_{Val66Met}-related functional network alterations are associated with worse global cognition and episodic memory.

METHODS

Participants

DIAN:

115 carriers of ADAD-causing mutations in genes *PSEN1* (n=82) & *PSEN2* (n=12) or *APP* (n=21), and 91 non-carrier (NC) siblings were included from DIAN data freeze 10.³¹ Beyond DIAN inclusion criteria, the current study required availability of *BDNF*_{Val66Met} genotype, resting-state fMRI, T1-structural MRI and cognitive assessments. No selection bias (i.e. demographic differences between the included subjects and excluded subjects) was found ($p>0.05$) for age, gender or education. As a marker of AD severity, we applied the estimated years from symptom onset (EYO), defined as the difference between a participants age at examination and the parental age of symptom onset for ADAD mutation carriers, as described previously.³⁶⁻³⁸ As an additional marker of amyloid pathology, we employed global PiB-PET SUVR scores (normalized to the whole cerebellum, available in a subsample of 100 mutation carriers and 87 non-carriers) provided by the DIAN core, where subjects were binarized as A β ⁺ at a SUVR threshold >1.31 following recommendations by the DIAN core. For details on PiB-PET assessment in DIAN, please refer to a previous publication.³⁹ *BDNF*_{Val66Met} genotyping was performed on whole-blood samples using the Infinium HumanExomeCore V1.0 Beadchip (Illumina, Inc.) at Washington University. Each participant provided written informed consent. Local ethical approval was obtained at each DIAN site.

DELCODE:

114 older adults (>60 years) were included from the German multicenter DELCODE cohort on sporadic AD (data freeze 1, N=366).³⁵ Beyond DELCODE inclusion criteria, subjects had to have available *BDNF*_{Val66Met} data, resting-state fMRI and T1-structural MRI, cerebrospinal fluid (CSF)-assessed A β -levels (A β 42/40 ratio) and cognitive assessments. Testing for selection bias yielded no significant ($p>0.05$) differences between the selected and excluded

subjects for age, gender and education. Subjects met classification criteria for cognitively normal (CN), SCD (i.e. normal cognitive performance and subjective cognitive decline between the last 6 months and 5 years), MCI or AD dementia, following previously described procedures.^{35, 37} Continuous CSF A β 42/40-ratio levels, i.e. a close correlate of brain A β -levels,⁴⁰ were used as a marker of AD pathology. For descriptive purposes A β -positivity was defined as CSF A β 42/40<0.1, following pre-established cut-points⁴¹ used within DELCODE.³⁷ *BDNF*_{Val66Met}-genotyping was performed on whole-blood samples using commercially available TaqMan probes (ThermoFischer). All subjects provided written informed consent, local ethical approval was obtained at each DELCODE site.

FACEHBI:

149 older adults (>50 years) were included from the Spanish monocentric FACEHBI cohort (n=214) collected at the Fundació ACE Alzheimer Treatment and Research Center in Barcelona. All subjects met research criteria for SCD as defined by the FACEHBI core (i.e. coexistence of subjective cognitive complaints defined as a score>7 on the memory failures in everyday life questionnaire,⁴² and normal performance in a comprehensive neuropsychological battery).⁴³ For a detailed sample description and inclusion criteria please see a previous publication.³⁴ For the current study, subjects were included based on availability of *BDNF*_{Val66Met} genotype, resting-state fMRI, T1-structural MRI, ¹⁸[F]-Florbetaben amyloid-PET and cognitive data. No significant differences (p>0.05) were found in age, gender or education between the selected and excluded subjects. AD pathology was assessed using continuous measures of global ¹⁸[F]-Florbetaben PET SUVR scores, that were additionally stratified into A β -positive/negative for descriptive purposes, using a standard uptake value ratio (SUVR) threshold >1.45, following previous recommendations.⁴⁴ The assessment of global A β -PET SUVR has been conducted by the FACEHBI core and was described in detail previously.⁴⁵ The *BDNF*_{Val66Met} genotype was extracted from GWAS data

performed on whole-blood samples with the Illumina Infinium Omni Express Exome-8v1.3 chip. All subjects provided written informed consent, ethical approval was obtained by the FACEHBI principal investigators.

Neuropsychology

As a primary measure of cognition, we used the Mini-Mental State Exam (MMSE)⁴⁶ which was consistently available in all samples, thus facilitating across cohort comparability. As a secondary measure of cognition, we used episodic memory as assessed by the delayed recall score of the Wechsler logical memory scale,⁴⁷ which was available in DIAN and DELCODE sample but not in FACEHBI.

MRI acquisition

All MRI scans were recorded on 3T scanners for DIAN and DELCODE, and a 1.5T scanner for FACEHBI. Within the multicenter studies DIAN or DELCODE, scanning protocols were harmonized across participating sites for each study. Here, we included resting-state fMRI and high-resolution T1-structural MRI scans. Detailed sequence parameters can be found in supplementary table 1.

Preprocessing of structural and resting-state fMRI

The same pre-established SPM12-based pipeline previously described by us,^{37, 48, 49} including motion correction, nuisance regression (motion, white-matter & CSF), censoring of high-motion frames, spatial normalization and smoothing was applied to each cohort (DIAN, DELCODE, FACEHBI) separately so no data was pooled across cohorts during the entire analyses. For details on MRI preprocessing please see supplementary methods.

Assessment of seed-based connectivity

We used a seed-based connectivity approach previously described by us^{37, 48, 49} in order to generate subject-specific Fisher z-transformed connectivity maps of hippocampal seeds and canonical resting-state networks (DMN, DAN, SAL, CON) for each subject from preprocessed resting-state fMRI data. For details on anatomical location of seed regions of interest (ROIs) and network topology please see figure 1 and supplementary methods.

Statistical analysis

Within each sample, we compared baseline group differences using two-sample t-tests or ANOVAS (for >2 groups) for continuous measures and Chi-squared tests for categorical measures. We further tested *BDNF*_{Val66Met} deviations from the Hardy-Weinberg Equilibrium within each sample. Significant seed-based connectivity in DIAN was mapped by conducting voxel-wise t-tests against zero on the subject-specific connectivity maps ($\alpha=0.001$ & FWE-cluster correction).

Next, we computed the voxel-wise interaction *BDNF*_{Val66Met} x EYO on seed-based connectivity maps in the 115 ADAD mutation-carriers (DIAN), controlling for gender, center, education, family affiliation and subject-specific grey matter volume of the seed-ROI that was extracted from spatially normalized and Jacobian-scaled grey matter images. The rationale to use EYO as a marker of disease severity in DIAN is that 1) EYO is a valid and reliable marker of global AD pathology and 2) that usage of EYO facilitates the comparability with previous studies in this cohort. In line with previous studies in DIAN we did not control for age in the model,^{37, 38} as age is highly correlated with EYO and may mask variance of interest and/or induce multicollinearity. Clusters showing a significant *BDNF*_{Val66Met} x EYO interaction ($\alpha=0.001$ & FWE-cluster correction at $\alpha=0.05$) were binarized to extract subject-specific mean connectivity values from individual connectivity maps (henceforth referred to

as *BDNF*-related connectivity) for later analyses. Using mean connectivity values, we further conducted confirmatory analyses in a subset of 100 mutation-carrier subjects with available global amyloid-PET SUVR levels instead of EYO, to ensure that our results were not specific for using EYO as a measure of ADAD severity. Specifically, we tested the interaction global amyloid-PET SUVR x *BDNF*_{Val66Met} on connectivity, controlling for gender, center, education, family affiliation and grey matter volume of the respective seed ROI.

After the discovery analysis in DIAN, cluster maps of significant EYO x *BDNF*_{Val66Met} interactions were forward applied to the DELCODE and FACEHBI groups to extract mean seed ROI to cluster connectivity values for validation analysis including the interaction *BDNF*_{Val66Met} x amyloid load on connectivity. As a measure of amyloid-load, we applied continuous CSF measures of the A β 42/40 ratio for DELCODE and continuous global amyloid-PET SUVR scores for FACEHBI. Due to the skewed distribution of global amyloid-PET SUVRs, the scores were Box-Cox transformed prior to analysis, after which there was no deviation from a normal distribution. Using separate linear models for DELCODE and FACEHBI, we then tested the A β x *BDNF*_{Val66Met} two-way interaction on connectivity, controlling for age, gender, education, ROI grey matter volume, ApoE4-status (as well as center and diagnosis for DELCODE).

We further extracted connectivity to clusters in the ADAD non-carrier group (DIAN), to assess potential *BDNF*_{Val66Met}-related connectivity changes in relatively young subjects unaffected by ADAD (i.e. *BDNF*_{Val66Met} x Age on connectivity, controlling for gender, family and grey matter volume of the respective seed ROI). The rationale for this analysis is, that the *BDNF*_{Val66Met} genotype may also have an effect on connectivity changes in subjects unaffected by ADAD.

Next, we tested separately within the ADAD subjects as well as in DELCODE and FACEHBI whether *BDNF*-related hippocampal connectivity (i.e. seed connectivity to clusters that showed a significant *BDNF*_{Val66Met} x EYO interaction in DIAN) moderated AD-related

cognitive decreases. For DELCODE and FACEHBI, we used robust linear mixed-effects models, where we tested within each sample the two-way interaction between *BDNF*-related hippocampal-medio-frontal connectivity and amyloid on 1) episodic memory or 2) global cognition controlling for gender, and hippocampal volume. Due to the study design of DELCODE we additionally included diagnosis as a fixed effect and center as a random effect in the respective statistical models. MMSE values were box-cox transformed prior to analyses to minimize skew. For DIAN, analogous regression analyses were run using MMSE or episodic memory as dependent variables, testing the two-way interaction between hippocampal-medial-frontal connectivity and EYO, controlling for family affiliation (to control for shared genetic background among DIAN family members), gender and hippocampal volume.

All voxel-wise analyses were computed in SPM12 and restricted to group-specific grey matter masks. Statistical analyses were conducted using *R* statistical software. Effects were considered significant when meeting a two-tailed alpha threshold of $\alpha=0.05$.

RESULTS

See table 1 for sample characteristics. Patterns of significant seed-based connectivity in the DIAN discovery sample are shown in Figure 1. No $BDNF_{Val66Met}$ deviations from Hardy-Weinberg equilibrium were found.

Discovery analysis: Interaction $BDNF_{Val66Met}$ x EYO on hippocampal connectivity in ADAD (DIAN)

We found a significant $BDNF_{Val66Met}$ x EYO interaction on connectivity between the right hippocampus and the bilateral medial-frontal cortex (henceforth referred to right hippocampal-medial-frontal connectivity), a key region of the DMN (MNI: x=-2, y=33, z=14, $t(108)=4.77$, blue and purple areas in Fig. 2A). When illustrating the interaction effect using mean connectivity values of that cluster, $BDNF_{Val}$ was associated with relatively stable right hippocampal-medial-frontal connectivity levels across EYO, whereas $BDNF_{Val66Met}$ was associated with a decline in right hippocampal-medial-frontal connectivity across EYO (Fig. 2B). For the left hippocampus seed ROI, the $BDNF_{Val66Met}$ x EYO interaction was also detected for the bilateral medial-frontal cortex (Fig 2E, MNI: x=4, y=49, z=-10, $t(109)=3.93$), where $BDNF_{Val}$ was associated with relatively preserved connectivity across EYO compared to $BDNF_{Val66Met}$ (Figure 2F). For the sake of consistency with the subsequent validation analyses in DELCODE and FACEHBI using continuous biomarkers of A β as the marker of AD pathology (see below), we repeated the analyses using global amyloid-PET levels instead of EYO as the predictor variable in a subset of 100 ADAD subjects with available PiB-PET. Results of the regression analysis showed a congruent interaction between global amyloid-PET SUVR and $BDNF_{Val66Met}$ on both left ($t(94)=3.074$, $\beta/SE=0.066/0.021$, $p=0.002$) and right ($t(94)=3.873$, $\beta/SE=0.083/0.021$, $p<0.001$) hippocampus-medial-frontal connectivity.

No Age x $BDNF_{Val66Met}$ interaction effects for right or left hippocampal connectivity were detected in the ADAD non-carriers, suggesting specificity for ADAD. Also, no main effect of

age on hippocampal connectivity was found in this group, suggesting that hippocampus connectivity remains relatively stable across age in the absence of ADAD. This view is further confirmed by an exploratory assessed ADAD-mutation (i.e. carriers vs. non-carriers) x age interaction on both left ($t(196)=-2.087$, $b/se=-0.005/0.002$, $p=0.038$) and right ($t(196)=-2.201$, $b/se=-0.006/0.003$, $p=0.029$) hippocampus-medial-frontal connectivity, when controlling for gender, seed ROI volume, family and $BDNF_{Val66Met}$. Here, ADAD mutation carriers show decreasing connectivity across age (which is highly correlated with EYO) while non-carriers remain relatively stable. These results suggest that the hippocampus-medial-frontal connectivity decreases as observed in the ADAD group are driven by AD pathology and do not reflect normal age-related connectivity changes.

In addition, none of the other resting-state networks (DMN, DAN, SAL, CON) showed a significant $BDNF_{Val66Met}$ x EYO interaction, both in ADAD mutation carriers and non-carriers (where we used age instead of EYO).

Validation analyses: $BDNF_{Val66Met}$ and hippocampal connectivity in subjects with sporadic A β pathology (DELCODE and FACEHBI).

To validate our findings of the association between $BDNF_{Val66Met}$ and hippocampal-medial-frontal connectivity in ADAD, we assessed the interaction $BDNF_{Val66Met}$ x A β on hippocampus-medial-frontal connectivity (Figures 2A&E) in the independent DELCODE and FACEHBI samples. Here, we employed A β -levels to assess AD severity, since A β is the defining feature of sporadic AD pathology in the mostly non-demented DELCODE and FACEHBI subjects.

By considering the clinical spectrum of CN, SCD, MCI, and AD dementia in DELCODE, we found a significant $BDNF_{Val66Met}$ x CSF A β 42/40 interaction on both right hippocampal-medial-frontal ($\beta/SE=-0.404/0.179$, $p=0.026$; Figure 2C) and left hippocampal-medial-frontal connectivity ($\beta/SE=-0.355/0.169$, $p=0.037$; Figure 2G), where $BDNF_{Val}$ was associated with

more stable connectivity across a decreasing CSF A β 42/40 ratio compared to *BDNF*_{Val66Met}. In FACEHBI (SCD subjects), significant *BDNF*_{Val66Met} x global amyloid-PET SUVR interactions were observed on right hippocampal-medial-frontal (β /SE=0.301/0.170, p =0.039; Figure 2D) and left hippocampal-medial-frontal connectivity (β /SE=0.333/0.195, p =0.045; Figure 2H). In exploratory analyses, no *BDNF*_{Val66Met} x global amyloid-PET SUVR interactions were found for any of the other functional networks in both DELCODE or FACEHBI. These results suggest congruent effects of *BDNF*_{Val66Met} on hippocampal connectivity in all three samples. Regression results are summarized in table 2.

BDNF_{Val66Met}-related hippocampal-medial-frontal connectivity moderates the effect of AD-severity on cognition

We next tested whether *BDNF*-related hippocampus-medial-frontal connectivity was associated with cognition. In DIAN, ADAD mutation carriers showed for both right and left hippocampal-medial-frontal connectivity (i.e. Fig 2A&E) a significant two-way interaction with EYO on MMSE, when controlling for gender, education, family affiliation, respective hippocampal volume and center (right: β /SE=0.202/0.047, p <0.001, left: β /SE=0.177/0.068, p =0.009). As hypothesized, individuals with lower right and left hippocampal-medial-frontal connectivity showed steeper MMSE decreases across EYO than individuals with higher FC. A hippocampal-medial-frontal connectivity x EYO interaction was also detected for logical memory recall (right: β /SE=0.144/0.071, p =0.045, Figure 3B; left: β /SE=0.160/0.077, p =0.041), with worse memory impairment at lower hippocampal-medial-frontal connectivity. In DELCODE, we found a significant two-way interaction such that individuals with lower right hippocampal-medial-frontal (β /SE=-0.222/0.080, p =0.007; Figure 3C) or left hippocampal-medial-frontal connectivity (β /SE=-0.205/0.079, p =0.011) showed stronger MMSE reductions as a function of more abnormal CSF-A β 42/40-levels, controlled for

diagnosis among other potentially confounding variables. All results remained consistent when additionally controlling for ApoE4 status. No interaction was found, however, for logical memory (Figure 3D, supplementary table 2). In the FACEHBI sample, no hippocampal-medial-frontal connectivity x global amyloid-PET SUVR two-way interaction effects on MMSE were found, probably due to lack of sufficient variance of cognitive performance in this cognitively normal SCD group.

DISCUSSION

Our major finding, observed across three independent cohorts, was that $BDNF_{Val66Met}$ was associated with stronger AD pathology-related decreases of hippocampal-medial-frontal connectivity. Furthermore, we found that lower hippocampal-medial-frontal connectivity was associated with stronger reductions in global cognition in both ADAD and symptomatic sporadic AD. Thus, our results suggest that the variation in $BDNF_{Val66Met}$ moderates the effect of AD pathology on hippocampus functional connectivity. Although the moderating effects of $BDNF_{Val66Met}$ -related hippocampus connectivity on cognition were not strong in the current study, our results provide preliminary evidence that hippocampal-mediofrontal-connectivity alterations linked to $BDNF_{Val66Met}$ may contribute to global cognitive impairment in AD.

In the current study, the association between $BDNF_{Val66Met}$ and decreased hippocampal-medial-frontal connectivity has been observed across different samples including non-symptomatic and symptomatic (MCI and dementia) AD stages, suggesting robust effects of $BDNF_{Val66Met}$ on regional hippocampal connectivity alterations across the AD spectrum. These results remained after accounting for hippocampus atrophy, suggesting that the association between $BDNF_{Val66Met}$ and hippocampal connectivity alterations cannot be fully explained by hippocampal neuronal loss. Strikingly, the association between $BDNF_{Val66Met}$ and stronger connectivity decreases was confined to the hippocampal networks since exploratory analyses of other major functional networks did not yield any significant effects. These findings are consistent with previous reports suggesting that $BDNF$ -related alterations occur specifically in the hippocampus rather than other brain regions. In healthy subjects, $BDNF_{Val66Met}$ was associated with reduced hippocampal activation during an encoding task, but showed no effect on a wider task-activated cortical network.⁵⁰ Furthermore, in ADAD, more pronounced FDG-PET hypometabolism within the hippocampus, but not precuneus, was observed in $BDNF_{Val66Met}$ -carriers compared to $BDNF_{Val}$ homozygotes.²⁰ Together, these results suggest that $BDNF_{Val66Met}$ is specifically associated with hippocampal alterations. Given that the

hippocampus is among the earliest regions to be affected by AD,^{51, 52} showing early connectivity decreases,^{32, 53} the design of the current study may have favored statistical power to detect *BDNF*_{Val66Met} effects specifically within the hippocampus as most subjects had only mild AD. Strikingly, the moderating effects of *BDNF*_{Val66Met} on the association between AD severity and hippocampal connectivity decreases were strongest in the ADAD group, as compared to the sporadic AD groups. This pronunciation of effects in ADAD might be due sample specific effects in the DIAN cohort. Thus, the effect size in the validation samples reflects probably a more likely approximation of the true effect size. Here, a large-scale study would be needed in order to conduct sensitivity analyses of any potential factors that may influence the observed effect size.

Connectivity between the hippocampus and medial frontal cortex is supported anatomically by direct neuronal connections.⁵⁴ Major mono-synaptic bidirectional connections between the orbitofrontal cortex and the peri- and entorhinal cortices, i.e. a major connection to the hippocampus, are constituted by the uncinate fasciculus as found in both post-mortem tracer studies and in-vivo diffusion-tensor imaging studies.⁵⁵ Functional imaging studies have shown that hippocampal-orbitofrontal connections are part of a network that is activated during cognitive tasks tapping episodic⁵⁶⁻⁵⁸ and working memory.^{59, 60} Alterations in hippocampal-prefrontal connectivity have been previously associated with reduced episodic- or working memory in AD^{55, 61, 62} or psychiatric diseases including schizophrenia,^{63, 64} suggesting that this anatomically supported functional network is critical for a wider range of cognitive abilities. We found that *BDNF*_{Val66Met}-related hippocampal-medial-frontal connectivity was associated with greater impairment in episodic memory and global cognition in ADAD, and stronger associations between A β (as measured by CSF A β 42/40) and impaired global cognition in a group covering the AD spectrum (DELCODE). However, no effect of *BDNF*_{Val66Met}-related hippocampal-medial-frontal connectivity on the association between A β (as assessed by amyloid-PET) and global cognition was found in subjects with

SCD (FACEHBI). The variability of findings between studies may be due to small variability in cognitive performance and AD-related cognitive changes in SCD, requiring larger sample sizes to detect any small effects of *BDNF*_{Val66Met} in cognitively normal subjects. Together, the current results suggest that *BDNF*_{Val66Met} enhances the vulnerability of this hippocampal-frontal memory network to the effects of A β and may thus worsen cognitive decline in symptomatic AD.

While we caution that the current results do not provide evidence for causal effects of *BDNF* genetic variants on functional connectivity, we encourage future studies to examine the mechanisms that underlie the potential effects of *BDNF*_{Val66Met} in AD. One possibility is that *BDNF*_{Val66Met} is associated with disturbed BDNF secretion, since preclinical *in-vitro* and *in-vivo* studies showed that *BDNF*_{Val66Met} is associated with impaired intracellular BDNF trafficking, entailing decreased synaptic BDNF levels.¹⁴ Since LTP may enhance connectivity between neural populations detectable by fMRI⁶⁵ and *BDNF*_{Val66Met}-related reduced BDNF bioavailability impairs LTP,¹⁵ *BDNF*_{Val66Met} may contribute to higher susceptibility of hippocampal connectivity to detrimental A β -effects. Note that we detected no difference between *BDNF*_{Val66Met} carriers and *BDNF*_{Val}-carriers in A β -biomarker levels. These findings are consistent with previous reports of the absence of altered *BDNF*_{Val66Met} effects on CSF A β ₁₋₄₂ in ADAD^{20, 21} or sporadic AD.^{17, 66} These results are also in line with previous findings in transgenic AD mouse-models, where lentiviral *BDNF*-gene delivery reduced cell loss and improved learning performance, but did not alter A β -burden.¹³ Similarly, reduced BDNF-levels were found in the P301L transgenic mouse model of tau pathology, where adeno-virus induced restoration of BDNF-levels alleviated synaptic degeneration and spatial memory deficits, but did not attenuate tau pathology.¹² Together, these findings suggest that BDNF is not associated with alterations in the primary pathology itself, rather lower BDNF (as proxied by *BDNF*_{Val66Met}) may be associated with enhanced susceptibility of hippocampus connectivity to the effects of AD pathology.

For drawing conclusions based on the current results, some caveats should be considered. We caution that *BDNF*_{Val66Met} effects on BDNF-levels in the brain of AD patients is not clear, and we did not assess BDNF protein levels directly. Previous studies reported decreased brain BDNF mRNA levels in AD mouse models⁶⁷ and AD patients¹⁰ as well as decreases of CSF-derived BDNF-levels in AD patients, but CSF or serum BDNF-levels were not altered by *BDNF*_{Val66Met}.^{68,69} The latter studies, however, included mostly healthy controls and only few MCI and AD cases, rendering the results difficult to interpret. *BDNF*_{Val66Met} effects on CSF or serum-derived BDNF-levels in MCI and AD remain to be investigated in future studies.

It should be acknowledged that we included a total of three different international studies using slightly different inclusion/exclusion criteria, MRI hardware and scanning protocols, which were not a priori harmonized across studies and can thus be considered a “naturalistic” sample of AD subjects. However, the heterogeneity in the sampling may also entail increased variability in the variable measurements and limited comparability. For instance, the FACEHBI 1.5T scanning protocol is potentially less sensitive to detect BOLD signal changes compared to the DIAN and DELCODE 3T scanning protocols.⁷⁰ In order to enhance comparability between studies, we applied harmonized MRI preprocessing and analysis pipelines and selected uniformly available cognitive tests. Importantly, however, rather than pooling data across all samples, where variability of the data may hamper the estimation of the regression parameters, we used a cross-validation approach in order to test the robustness and external validity of our initial analysis in the ADAD sample. Thus, our highly consistent findings across cohorts reduce the likelihood of our results being driven by technical assessment procedures or selection criteria.

A further potential limitation is that ADAD and sporadic AD may show slight differences in disease development. While ADAD and sporadic AD share core neuropathological and clinical features⁷¹ and functional network changes⁷² (for a review, see⁷³), several differences have been reported as well: Compared sporadic AD, ADAD is associated with an earlier

symptom onset,⁷⁴ stronger striatal amyloid accumulation,⁷⁵ higher likelihood of non-memory cognitive deficits,⁷⁴ higher prevalence of psychosis and hallucinations⁷¹, spastic paraparesis and motor symptoms.^{76, 77} Given such differences between ADAD and sporadic AD, it is possible that our approach of using the data from ADAD as the discovery sample may entail missing some *BDNF*_{Val66Met}-associated brain network effects that are specific to sporadic AD. To address this caveat, we tested in exploratory secondary analyses the interaction effect of *BDNF*_{Val66Met} x A β on each resting-state network in both the DELODE and FACEHBI studies. The absence of any interaction effect of the *BDNF*_{Val66Met} genotype by A β biomarker levels on any network other than the hippocampal network supports the conclusion that the effects of *BDNF*_{Val66Met} on exclusively hippocampal connectivity are consistent across samples, despite potential clinic-pathological differences between ADAD and sporadic AD.

Lastly, we did not assess tau pathology in the current study. Tau deposition emerges first in the entorhinal cortex and hippocampus⁷⁸ and has been shown to be correlated with cognitive performance and hippocampus connectivity in humans.⁷⁹ Given previous preclinical evidence that BDNF may modulate the neurotoxic effects of tau,¹² *BDNF*_{Val66Met} may also alter the effect of tau on hippocampus function. Additionally, previous studies of ADAD have reported that *BDNF*_{Val66Met}-carriers show faster CSF tau and p-tau₁₈₁ increases.²¹ The recent development of tau-PET will allow future studies to assess regional tau levels in the brain,⁸⁰ and provide an opportunity to test the potential modulating effects of *BDNF*_{Val66Met} on hippocampus connectivity in future studies.

Conclusions

In summary, our results suggest that *BDNF*_{Val66Met} is associated with increased impairment of hippocampal-medial-frontal connectivity cortex in the presence of AD pathology.

Our current results are consistent with previous studies suggesting that *BDNF*_{Val66Met} may moderate the neurotoxic effects of A β on hippocampal connectivity and memory impairment

in AD. A critical future question will be, whether beneficial effects of *BDNF*_{Val66Met} show a disease stage-dependent peak i.e. at the preclinical, prodromal or dementia stage of AD. We thus encourage future studies to assess this question as soon as large enough data become available for each subgroup. Our results have clinical implications: BDNF-levels are modifiable in humans and could thus become a promising treatment target to enhance resilience against the impact of neurotoxic primary AD pathology.⁹ Alterations of hippocampal connectivity can be a potential outcome measure to assess the effect of BDNF-targeting drugs in AD. Increased BDNF-levels after aerobic exercise have been observed in subjects with neurological disorders,⁸¹ and this may mediate the effect of exercise on enhanced synaptic plasticity and hippocampus-dependent learning.⁸² Enhancing BDNF-levels in the brain may thus constitute a promising secondary-preventive approach to attenuate AD-effects on brain integrity and cognitive function.^{9, 83}

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Table 1: Sample characteristics

DIAN	MC (n=115)	NC (n=91)			p-value
Age	38.97 (10.48)	39.19 (10.67)			0.882
Gender (f/m)	74/41	56/35			0.678
BDNF _{Val66Met} (Met/Val)	44/71	24/67			0.071
Estimated years from symptom onset (EYO)	-7.17 (11.15)	-9.03 (11.22)			0.237
Global PiB-PET SUVR ^a	1.99 (1.15)	1.07 (0.19)			<0.001
Aβ-status (pos./neg./NA) ^b	57/43/15	0/87/4			<0.001
LM delayed recall	10.29 (6.46)	13.98 (4.12)			<0.001
MMSE	26.84 (5.1)	29.23 (1.25)			<0.001
DELCODE	CN (n=47)	SCD (n=39)	MCI (n=17)	AD dementia (n=11)	p-value
Age	67.79 (4.63)	71.46 (5.18)	71.76 (5.53)	74.00 (4.2)	<0.001
Gender (f/m)	30/17	18/21	4/13	8/3	0.014
BDNF _{Val66Met} (Met/Val)	18/29	15/24	5/12	5/6	0.851
CSF Aβ42/40	0.1 (0.02)	0.09 (0.03)	0.08 (0.03)	0.05 (0.02)	<0.001
Aβ-status (pos./neg.) ^c	21/26	25/14	11/6	11/0	0.019
ApoE4-status (pos./neg.)	10/37	11/28	5/12	8/3	0.010
LM delayed recall	14.91 (4.46)	13.59 (3.27)	8.41 (6.09)	3.09 (4.41)	<0.001
MMSE	29.55 (0.90)	29.21 (0.92)	27.71 (1.90)	25.00 (4.05)	<0.001
FACEHBI	SCD (n=149)				
Age	65.81 (7.16)				
Gender (f/m)	92/57				
BDNF _{Val66Met} (Met/Val)	67/82				
Global Florbetaben- PET SUVR	1.23 (0.14)				
Aβ-status (pos./neg.) ^d	14/135				
ApoE4-status (pos./neg.)	41/108				
MMSE	29.2 (0.94)				

a = Subsample of 100 MC and 87 NC, b = A β positive defined as global PiB-PET SUVR < 1.31, c = A β positive defined as a CSF A β 42/40 ratio < 0.1⁴¹; d = A β positive defined as a global SUVR > 1.45⁴⁴

Table 2: linear models for the interaction Amyloid x BDNF on Hippocampal-medial-frontal connectivity in DELCODE and FACEHBI

	Model terms	B/SE	T	p
DELCODE- Hipp-R ^a	CSF A β 42/40 x <i>BDNF</i> _{Val66Met}	-0.404/0.179	-2.261	0.026
	CSF A β 42/40	0.373/0.156	2.393	0.018
	<i>BDNF</i> _{Val66Met}	0.217/0.184	1.176	0.242
DELCODE- Hipp-L ^a	CSF A β 42/40 x <i>BDNF</i> _{Val66Met}	-0.355/0.169	-2.106	0.037
	CSF A β 42/40	0.470/0.147	3.195	0.002
	<i>BDNF</i> _{Val66Met}	0.222/0.174	1.270	0.207
FACEHBI Hipp-R ^b	Global Amyloid SUVR x <i>BDNF</i> _{Val66Met}	0.301/0.170	2.040	0.043
	Global Amyloid SUVR	-0.186/0.123	-1.455	0.148
	<i>BDNF</i> _{Val66Met}	-0.195/0.168	-1.159	0.248
FACEHBI Hipp-L ^b	Global Amyloid SUVR x <i>BDNF</i> _{Val66Met}	0.333/0.195	2.089	0.039
	Global Amyloid SUVR	-0.274/0.177	-1.553	0.123
	<i>BDNF</i> _{Val66Met}	0.075/0.167	0.655	0.514

a=Linear model controlled for age, gender, education, hippocampus volume, diagnosis, ApoE4 status and center

b= Linear model controlled for age, gender, education, hippocampus volume and ApoE4 status

Hipp-R = Right Hippocampal-medial-frontal connectivity; Hipp-L = Left Hippocampal-medial-frontal connectivity

Figure Legends:

Figure 1: Surface renderings of significant seed-based connectivity ($p < 0.001$, FWE cluster corrected at $p < 0.05$) in the DIAN discovery sample for the primary hippocampal seed ROIs and the secondary seed ROIs to derive connectivity for the control network (CON), dorsal attention network (DAN), default mode network (DMN) and salience network (SAL).

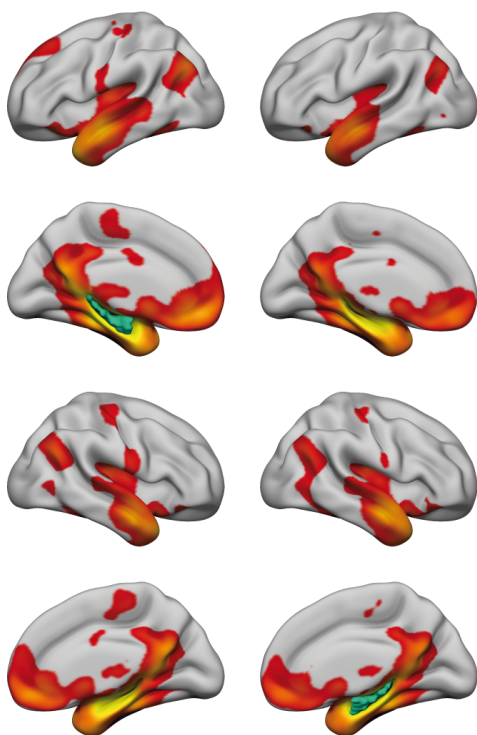
Figure 2: Results of the voxel-wise interaction analysis of $EYO \times BDNF_{Val66Met}$ on hippocampal connectivity in the DIAN MC subjects for the left (A&B) and right (E&F) hippocampus seed ROI (i.e. discovery). In Panels A&E, red areas correspond to regions showing significant seed-based hippocampal connectivity, while purple and blue regions indicate the boundaries of the significant $EYO \times BDNF_{Val66Met}$ interaction. Validation analyses of the $BDNF_{Val66Met} \times A\beta$ interaction in DELCODE and FACEHBI using the DIAN MC derived medial-frontal ROI are shown in panels C&E for DELCODE and G&H for FACEHBI. DIAN-MC = mutation carriers with autosomal dominant AD from DIAN.

Figure 3: Scatterplots illustrating the interaction between right (A-D) and left (E-H) hippocampus to medial-frontal connectivity and AD severity (i.e. EYO or CSF $A\beta_{42/40}$ ratio) on cognition. Color groupings were based on median split and are for illustrational purposes only, since the underlying linear mixed models were computed using continuous measures. DIAN-MC = mutation carriers with autosomal dominant AD from DIAN.

Primary ROIs

Hipp-L

Hipp-R



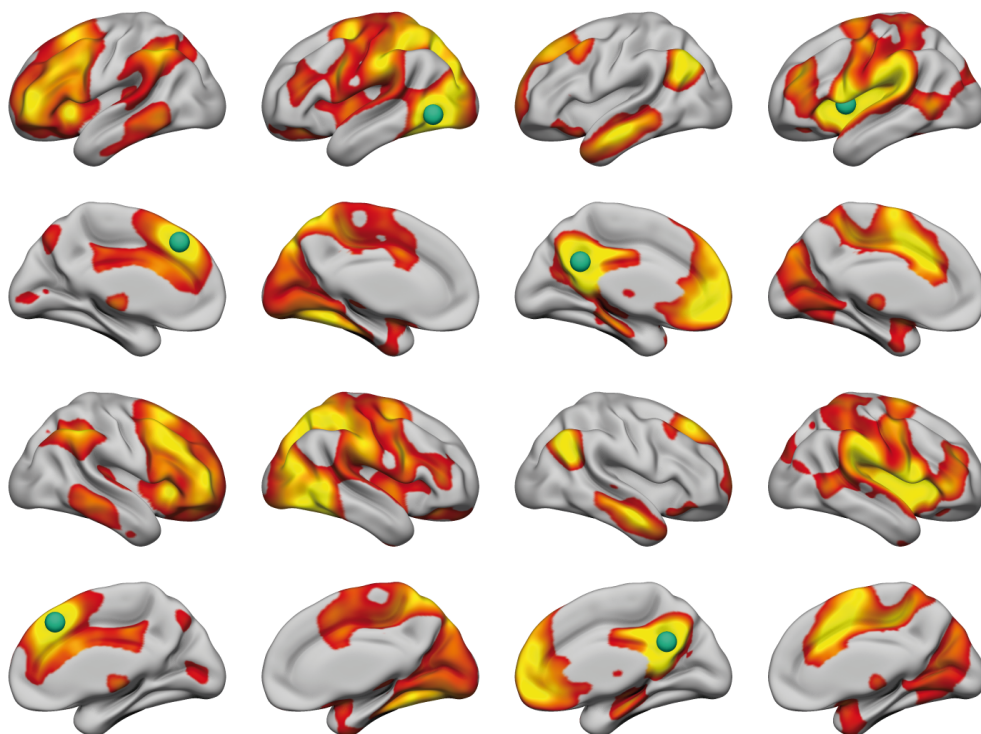
Secondary ROIs

CON

DAN

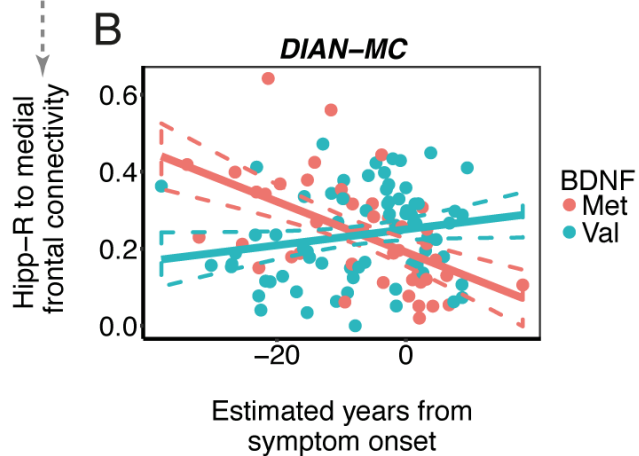
DMN

SAL

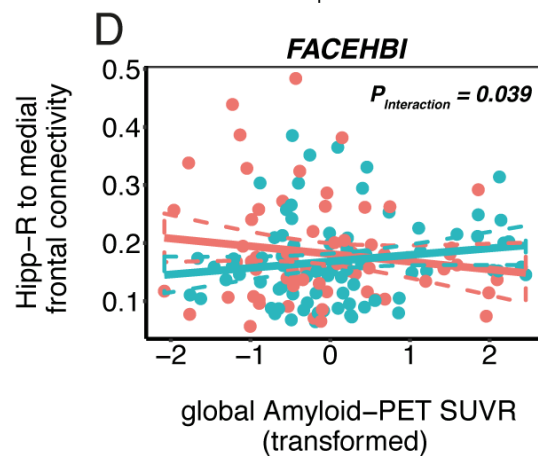
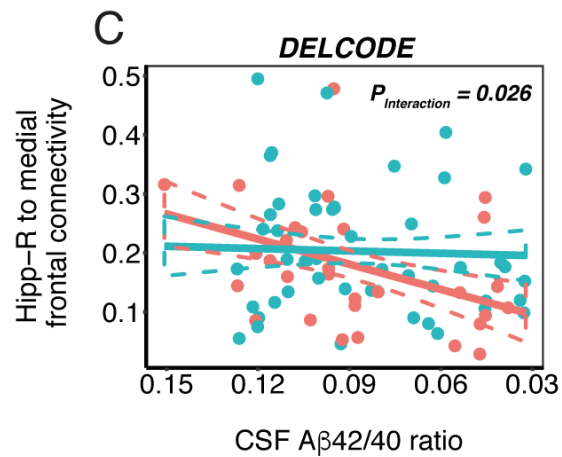


Right Hippocampus - Discovery

A *Voxel-wise interaction:*
Hipp-R FC ~ BDNF-Val66Met x EYO + Hipp-R Volume + Gender + Site + Education + Family
 $p < 0.001$, FWE cluster corrected at $p < 0.05$

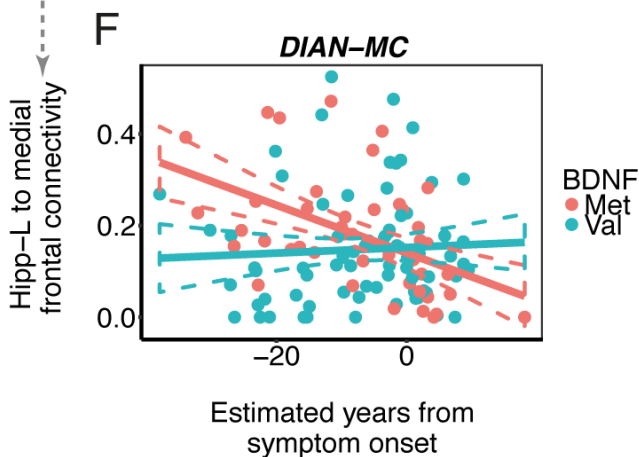
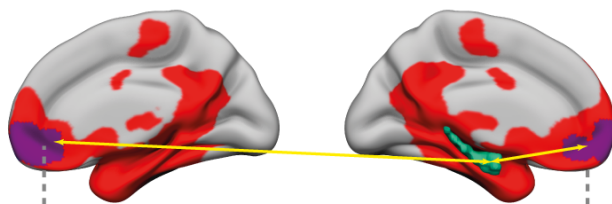


Validation

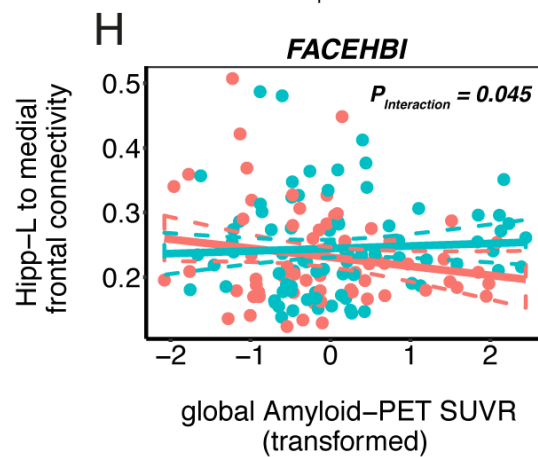
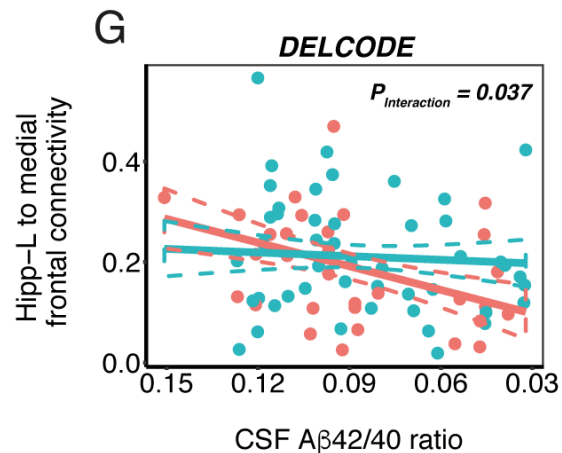


Left Hippocampus - Discovery

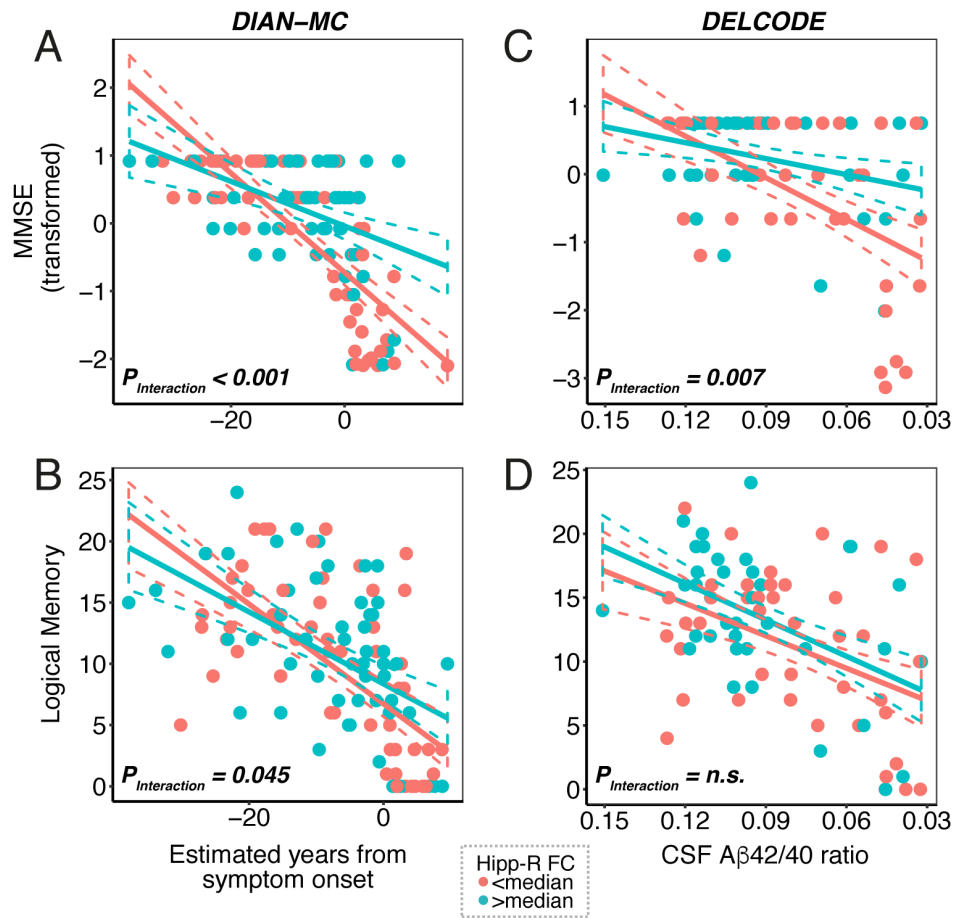
E *Voxel-wise interaction:*
Hipp-L FC ~ BDNF-Val66Met x EYO + Hipp-R Volume + Gender + Site + Education + Family
 $p < 0.001$, FWE cluster corrected at $p < 0.05$



Validation



Right Hippocampus



Left Hippocampus

